Generation of protein adducts with malondialdehyde and acetaldehyde in muscles with predominantly type I or type II fibers in rats exposed to ethanol and the acetaldehyde dehydrogenase inhibitor cyanamide[1–3]

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ABSTRACT

Background: Alcoholic myopathy is known to primarily affect type II muscle fibers (glycolytic, fast-twitch, anaerobic), whereas type I fibers (oxidative, slow-twitch, aerobic) are relatively protected.

Objective: We investigated whether aldehyde-derived adducts of proteins with malondialdehyde and acetaldehyde are formed in muscle of rats as a result of acute exposure to ethanol and acetaldehyde. The differences between type I muscle, type II muscle, and liver tissue were also assessed.

Design: The formation and distribution of malondialdehyde- and acetaldehyde-protein adducts were studied with immunohistochemistry in soleus (type I) muscle, plantaris (type II) muscle, and liver in 4 groups of rats. The different groups were administered saline (control), cyanamide (an acetaldehyde dehydrogenase inhibitor), ethanol, and cyanamide + ethanol.

Results: Treatment of rats with ethanol and cyanamide + ethanol increased the amount of aldehyde-derived protein adducts in both soleus and plantaris muscle. The greatest responses in malondialdehyde-protein and acetaldehyde-protein adducts were observed in plantaris muscle, in which the effect of alcohol was further potentiated by cyanamide pretreatment. Malondialdehyde- and acetaldehyde-protein adducts were also found in liver specimens from rats treated with ethanol and ethanol + cyanamide; the most abundant amounts were found in rats given cyanamide pretreatment.

Conclusions: Acute ethanol administration increases protein adducts with malondialdehyde and acetaldehyde, primarily in type II muscle. This may be associated with the increased susceptibility of anaerobic muscle to alcohol toxicity. Higher acetaldehyde concentrations exacerbate adduct formation, especially in type II–predominant muscles. The present findings are relevant to studies on the pathogenesis of alcohol-induced myopathy. *Am J Clin Nutr* 2002;76:668–74.

KEY WORDS Alcohol, myopathy, skeletal muscle, ethanol metabolism, acetaldehyde, lipid peroxidation, alcoholic myopathy, alcoholism, muscle, fast-twitch muscle, slow-twitch muscle, type I muscle fiber, type II muscle fiber, protein adduct, malondialdehyde

INTRODUCTION

Alcoholic myopathy of the skeletal muscle is characterized clinically by muscle weakness, recurrent falls, and difficulties in gait (1–3). Such problems are found in up to two-thirds of chronic alcohol misusers. In histologic specimens, there is usually a preferential reduction in the diameter of type II (glycolytic, fast-twitch, anaerobic) muscle fibers, whereas type I (oxidative, slow-twitch, aerobic) fibers exhibit atrophy only in the severe forms of the disease and actually show a compensatory hypertrophy in the initial stages (4–8). Fiber atrophy may reflect loss of muscle tissue protein, because previous studies showed reductions in muscle myofibrillary myosin-heavy chains (assessed by measuring Ca^{2+}-ATPase activities) (9), urinary excretion of creatinine (10), muscle protein per unit DNA (11), midarm circumference (10), and muscle mass assessed with computed tomography (12). Reduced muscle strength, along with the reduced mass, is usually a focal lesion and may persist for several years after the individual stops consuming alcohol.

Several processes may contribute to the pathogenesis of alcoholic myopathy. These processes include membrane defects (13) as well as alterations in protein turnover (14, 15) and gene expression (16). The metabolic effects of ethanol include production of toxic metabolites and free radicals together with ethanol-induced oxidant stress. The process of increased lipid peroxidation leads to the production of malondialdehyde, which is able to bind to proteins by covalent modifications forming stable adducts (17, 18). Previous studies indicated that such processes may play a pivotal role in the pathogenesis of liver disease in alcoholics (18, 19).

We recently found evidence of acetaldehyde-protein adduct formation in muscles of rats fed ethanol for 6 wk according to the Lieber-DeCarli feeding protocol (20). However, with this ethanol exposure we were unable to show differences in the responses between the soleus muscle (predominantly type I fibers) and the plantaris muscle (predominantly type II fibers). The findings also

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did not show evidence of malondialdehyde-protein adduct formation in muscle, as analyzed with enzyme-linked immunosorbent assays of tissue homogenates. The former observation is at variance with the clinical findings showing that type II fibers are more susceptible to the effects of alcohol than are type I fibers (1). However, several studies suggested that the differences between the biochemistry of these 2 fiber types are discernible in the acute phase of alcohol exposure. For example, in young rats, ethanol-induced reductions in protein synthesis are much greater after an acute dose (14) than with chronic consumption (15), which is consistent with the development of tolerance.

In this study, we used immunohistochemical methods to examine adduct formation in the different muscle fiber types after acute exposure to ethanol. Both malondialdehyde- and acetaldehyde-protein adducts from muscle were assessed and were compared with those from liver specimens. To further explore the roles of ethanol and acetaldehyde, we also studied pretreatment of rats with cyanamide, which increases tissue and circulating acetaldehyde concentrations and stimulates lipid peroxidation.

MATERIALS AND METHODS

Animals

Male Wistar rats were obtained from Charles River (Margate, Kent, United Kingdom) at ≈60 g body weight. They consumed a commercial pelleted diet ad libitum; the diet contained 17.9% crude protein, 3.6% crude fiber, 57% carbohydrate, and 13.3 MJ/kg (CRM diet; Special Diets Services, Essex, United Kingdom). The rats were housed in cages in an air-conditioned (20–25 °C), humidified (40–60%) animal house with a 12 h light and 12 h dark cycle starting at 0800. After 1 wk of acclimatization, the rats weighed ≈150 g and were divided into the following 4 groups: saline + saline ($n = 8$; control group), cyanamide + saline ($n = 7$), saline + ethanol ($n = 8$), and cyanamide + ethanol ($n = 8$).

The dose of ethanol used was 75 mmol ethanol/kg body weight, and the dose of cyanamide was 0.5 mmol/kg body weight. Controls were injected with identical volumes of 0.15 mol NaCl/L. The experimental procedure involved an intraperitoneal injection (0.5 mL/100 g body weight) of either saline or cyanamide (pretreatment) followed by an intraperitoneal injection (1 mL/100 g body weight) of either saline or ethanol (treatment). Rats were killed by decapitation after a total time of 3 h (ie, 0.5 h of pretreatment and 2.5 h of treatment). The soleus and plantaris muscles were quickly dissected out for immunohistochemistry. A portion of liver was also obtained from each rat. Tissue specimens were paraffin-bedded for immunohistochemistry. All animals received humane care in compliance with the institutional guidelines (King's College, London) for the care and use of laboratory animals.

Preparation of antisera and immunohistochemistry

Protein-malondialdehyde modification was performed according to previously established methods by incubating freshly prepared LDL for 3 h at 37 °C with 0.5 mol malondialdehyde/L (18, 21, 22). Malondialdehyde was generated from malondialdehyde bis-dimethylacetal via acid hydrolysis. Polyvalent antisera against the modified protein were subsequently generated by immunizing rabbits with homologous protein modifications. The primary immunization consisted of intradermal injections of 500 mg malondialdehyde-modified lipoprotein suspended in phosphate buffered saline and Freund's complete adjuvant (Difco Laboratories, Detroit) in a 1:1 ratio. Booster injections of 250 mg antigen in Freund's incomplete adjuvant were given at 3–4-wk intervals. Antisera against the acetaldehyde-modified proteins were raised by immunizing rabbits with bovine serum albumin conjugated with 1 mmol acetaldehyde/L, prepared under reducing conditions as described previously (23, 24).

For immunohistochemistry, sections were stained by using the biotin-avidin complex method with an automated staining procedure according to the instructions of the manufacturer (Ventana Medical Systems, Tucson, Arizona). The primary antisera (diluted 1:200) was allowed to react for 30 min. After slide rinsing, the biotinylated secondary antibody was added and allowed to react for 8 min. Antigen-antibody binding was detected by using one reagent dispense of avidin-horseradish peroxidase conjugate followed by diaminobenzidine. The intensity of the staining was scored by 2 of the investigators (ON and SP) in a blinded fashion by using a scale of 0 (no reaction) to 5 (strong reaction). The stained sections were photographed with a Nikon Eclipse E600 microscope (Nikon, Tokyo).

Statistics

All data are expressed as means ± SEMs. Comparisons between groups were carried out by using analysis of variance with the Bonferroni method for multiple comparisons. Statistical analyses were performed with GRAPHPAD PRISM for WINDOWS (GraphPad Software Inc, San Diego). $P < 0.05$ was considered statistically significant.

RESULTS

Concentrations of ethanol and acetaldehyde

In this study, the blood concentrations of ethanol and acetaldehyde were not measured because we used a standard dosing protocol and we had measured these concentrations previously with this same protocol in identically sized rats. In this previous research, blood acetaldehyde concentrations (measured with HPLC) were ≈5 nmol/mL in control rats that were equivalent to the saline + saline group, ≈2500 nmol/mL in rats equivalent to the cyanamide + ethanol group, and ≈30 nmol/mL in ethanol-injected rats equivalent to the saline + ethanol group. Corresponding acetaldehyde values for the liver were 31, 1200, and 75 nmol/g for the 3 groups, respectively, and values for muscle were 51, 830, and 62 nmol/g, respectively. Blood ethanol concentrations were < 1, 46, and 33 mmol/L, respectively (25).

Immunohistochemical localization of aldehyde-derived adducts in muscle

Immunohistochemical stainings for malondialdehyde-protein adducts in the saline + saline rats and cyanamide + saline rats showed either negative results or weak positive signals consisting of sporadic subsarcolemmal or intracellular reactions (**Figure 1**). In the ethanol + saline rats, the staining was more intensive and showed a patchy, uneven distribution. Soleus muscle usually showed weaker reactions than did plantaris muscle. In plantaris muscle, a sarcolemmal reaction occurred in addition to intracellular staining. However, weak sarcolemmal staining was also found in the saline + saline group.

Staining for the acetaldehyde-protein adducts in soleus and plantaris muscle showed patterns of adduct distributions that were

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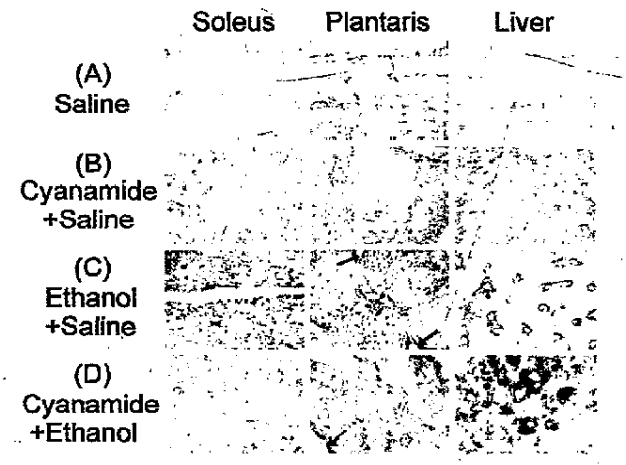

FIGURE 1. Immunohistochemical localization of malondialdehyde-protein adducts in soleus muscle (representing type I–predominant muscle), plantaris muscle (representing type II–predominant muscle), and liver tissue. The figure shows representative stainings for malondialdehyde-protein adducts in 4 groups of rats administered saline + saline for the pretreatment + treatment (A; control group), cyanamide + saline (B), ethanol + saline (C), or cyanamide + ethanol (D). The muscle sections of control animals are mainly negative or show weak subsarcolemmal or intracellular reactions. Muscle sections of ethanol-exposed rats show more intensive staining with a patchy, uneven distribution; staining is less intensive in soleus than in plantaris muscle. A distinct sarcolemmal component (indicated by arrows) and intracellular staining occurred in plantaris muscle. However, some sarcolemmal staining also occurred in control animals. The horizontal size of each panel within the figure represents 100 μm.

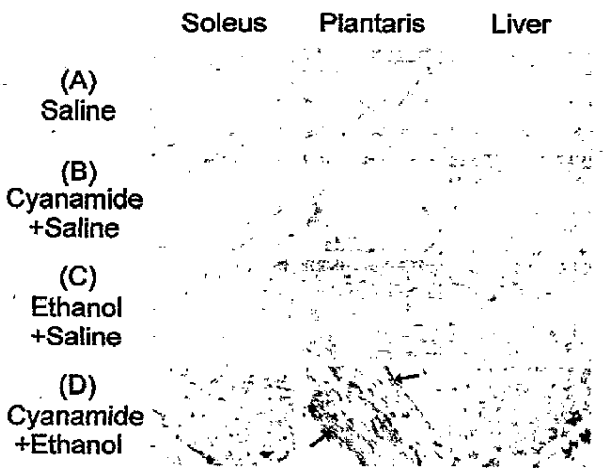

FIGURE 2. Immunohistochemical localization of acetaldehyde-protein adducts in soleus muscle (representing type I–predominant muscle), plantaris muscle (representing type II–predominant muscle), and liver tissue. The figure shows representative stainings in 4 groups of rats administered saline + saline for the pretreatment + treatment (A, control group), cyanamide + saline (B), ethanol + saline (C), or cyanamide + ethanol (D). The muscle sections of control animals are mainly negative or show weak subsarcolemmal or intracellular reactions. Muscle sections of ethanol-exposed rats show more intensive staining with a patchy, uneven distribution; staining is less intensive in soleus than in plantaris muscle. The arrows indicate the sarcolemmal staining component. The horizontal size of each panel within the figure represents 100 μm.

similar to those of the malondialdehyde-protein adducts, although the staining intensities varied between groups (**Figure 2**). The scoring data on the malondialdehyde- and acetaldehyde-protein adducts in soleus, plantaris, and liver tissue for the 4 groups of rats are summarized in **Figures 3**, **4**, and **5**, respectively.

Malondialdehyde- and acetaldehyde-protein adduct scores in muscle

Animals not exposed to ethanol

Mean malondialdehyde- and acetaldehyde-protein adduct scores in the groups given saline, cyanamide, or both but not given ethanol were not significantly different from each other in any of the tissues examined (Figures 3–5). However, the baseline amounts (ie, in the saline + saline group) of both types of adducts in plantaris muscle were higher than those in soleus muscle (Figures 3 and 4). Mean malondialdehyde-protein adduct scores for all animals without ethanol exposure (ie, the saline + saline group and cyanamide + saline group combined) were 1.37 ± 0.15 for plantaris muscle and 1.07 ± 0.10 for soleus muscle; the difference between these values was not significant. The acetaldehyde-protein adduct scores in the same combined group were also higher in plantaris muscle (1.67 ± 0.10) than in soleus muscle (1.45 ± 0.08), but again the difference was not significant.

Animals exposed to ethanol and cyanamide: malondialdehyde-protein adducts

In soleus muscle, malondialdehyde-protein adduct formation was significantly greater with ethanol administration ($P < 0.05$) than in rats exposed to saline only (Figure 3). However, increasing the acetaldehyde concentrations with cyanamide pretreatment was not found to potentiate this effect (Figure 3). In plantaris muscle, cyanamide pretreatment before ethanol exposure resulted in a potentiation of adduct generation, with significantly higher amounts than those in plantaris muscle of rats given saline only ($P < 0.01$; Figure 4).

The supposition that plantaris muscle developed more abundant amounts of malondialdehyde-protein adducts was also supported by the analysis of the soleus-to-plantaris adduct ratios in individual rats, an approach used previously to discern differences between these 2 distinct muscle types (14, 15). The mean (± SEM) soleus-to-plantaris ratios (n = 6–8) were as follows: saline + saline group, 0.853 ± 0.113; cyanamide + saline group, 0.847 ± 0.052; saline + ethanol group, 0.934 ± 0.090; and cyanamide + ethanol group, 0.633 ± 0.043. When these ratios were compared to analyze for significant differences between them, there was a significant difference between the ratios for the saline + ethanol and cyanamide + ethanol groups ($P < 0.05$). There were no significant differences between the saline + saline group and each of the other 3 groups.

Animals exposed to ethanol and cyanamide: acetaldehyde-protein adducts

The acetaldehyde-protein adduct scores in soleus muscle were not significantly affected by any of the treatments, ie, scores in the saline + saline group did not differ significantly from scores in the other 3 groups (Figure 3). In plantaris muscle, higher acetaldehyde-protein adduct scores were found after cyanamide + ethanol (+32%, $P < 0.05$; Figure 4) than after saline + saline.

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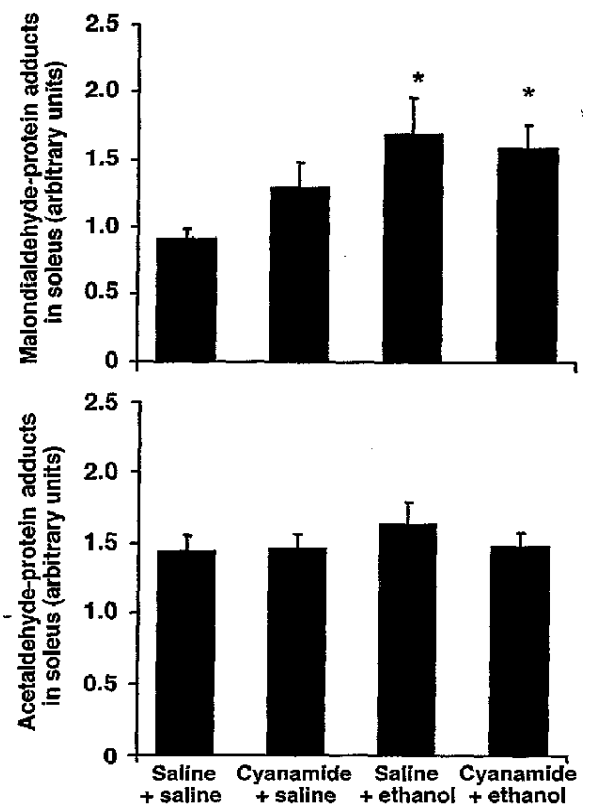

FIGURE 3. Mean (±SEM) staining intensity scores for malondialdehyde-protein adducts and acetaldehyde-protein adducts in soleus muscle (representing type I–predominant muscle) after acute ethanol administration with or without cyanamide (n = 6–8 rats/group). In the pretreatment phase, animals were injected with either saline (0.15 mol NaCl/L) or cyanamide (0.5 mmol/kg body weight) 30 min before administration of either saline or ethanol (75 mmol/kg body weight). At 2.5 h after the last injections, samples of soleus muscle were processed for immunohistochemical studies. *Significantly different from the saline + saline (control) group, $P < 0.05$.

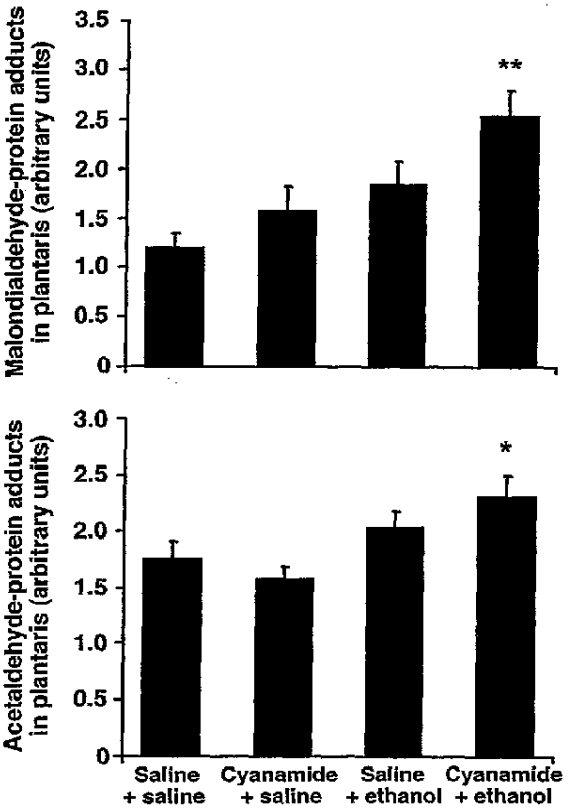

FIGURE 4. Mean (±SEM) staining intensity scores for malondialdehyde-protein adducts and acetaldehyde-protein adducts in plantaris muscle (representing type II–predominant muscle) after acute ethanol administration with or without cyanamide (n = 6–8 rats/group). In the pretreatment phase, animals were injected with either saline (0.15 mol NaCl/L) or cyanamide (0.5 mmol/kg body weight) 30 min before administration of either saline or ethanol (75 mmol/kg body weight). At 2.5 h after the last injections, samples of plantaris muscle were processed for immunohistochemical studies. *,**Significantly different from the saline + saline (control) group, $^{*}P < 0.05$, $^{**}P < 0.01$.

The mean (±SEM) soleus-to-plantaris ratios (n = 6–8) also showed greater sensitivity of plantaris muscle, as follows: saline + saline group, 0.858 ± 0,078; cyanamide + saline group, 0.871 ± 0.027; saline + ethanol group, 0.800 ± 0.055; and cyanamide + ethanol group, 0.671 ± 0.076. When these ratios were compared to analyze for significant differences between them, there was a significant difference between the ratios for the saline + saline and cyanamide + ethanol groups ($P < 0.05$). There were no significant differences between the saline + saline group and the other 2 groups or between the saline + ethanol and cyanamide + ethanol groups.

Malondialdehyde- and acetaldehyde-protein adduct scores in the liver

Malondialdehyde-protein adduct scores were significantly higher in liver specimens of rats given saline + ethanol than in rats given saline + saline (+70%, $P < 0.05$; Figure 5). The highest scores were observed in the cyanamide + ethanol group (+112%; $P < 0.001$). Although the acetaldehyde-protein adduct scores were also higher in rats exposed to ethanol, a significant difference when compared with the saline + saline group was obtained only after cyanamide pretreatment (+53%, $P < 0.05$). However, there was no statistically significant difference between the saline + ethanol and cyanamide + ethanol groups.

DISCUSSION

The present findings show the preferential formation of protein adducts with malondialdehyde and acetaldehyde in type II muscle fibers as a result of acute ethanol exposure. Malondialdehyde-protein adducts were detected by using antibodies that were shown previously to recognize malondialdehyde-derivatized lysine epitopes (18, 21, 22). The antibodies against the acetaldehyde-protein adducts were shown to detect primarily the reduced acetaldehyde-derived condensates in proteins independent of the nature of the carrier protein (23). However, adducts prepared

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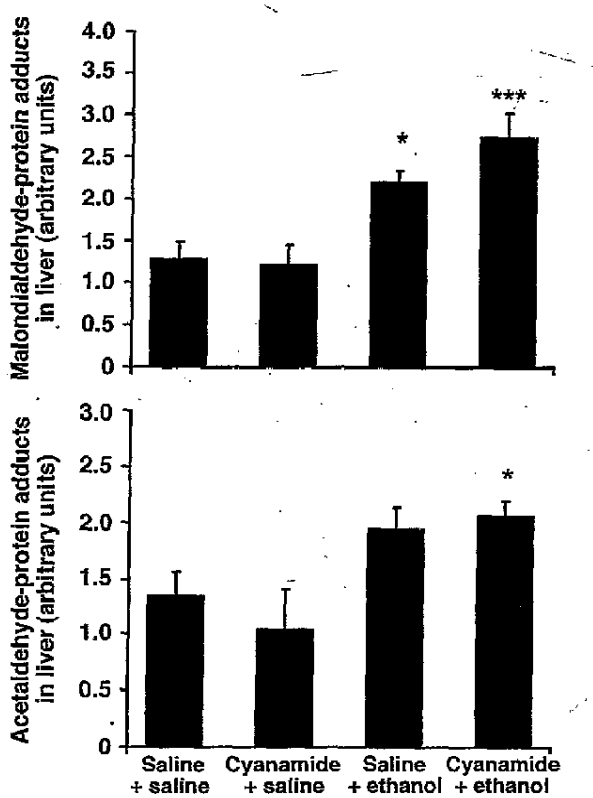

FIGURE 5. Mean (±SEM) staining intensity scores for malondialdehyde-protein adducts and acetaldehyde-protein adducts in liver tissue after acute ethanol administration with or without cyanamide (n = 6–8 rats/group). In the pretreatment phase, animals were injected with either saline (0.15 mol NaCl/L) or cyanamide (0.5 mmol/kg body weight) 30 min before administration of either saline or ethanol (7.5 mmol/kg body weight). At 2.5 h after the last injections, samples of liver tissue were processed for immunohistochemical studies. *, ***Significantly different from the saline + saline (control) group, $^{*}P < 0.05$, $^{***}P < 0.001$.

under unreduced conditions may also react with such antibodies. In vitro, acetaldehyde was shown previously to form stable adducts with muscle proteins, such as actin (26).

In the present study, some positive reactions to acetaldehyde-protein adducts occurred in skeletal muscle of rats with saline or cyanamide treatment, and this is in accordance with the supposition that muscles of control animals may contain acetaldehyde-protein adducts, protein adducts, or both. The evidence supporting this includes the following: *1*) Western blot analysis of proteins from soleus and plantaris muscles after a 6-wk ethanol feeding experiment showed some positive reactions in control animals (G Alexander, TJ Peters, and VR Preedy, unpublished observations, 2002), *2*) acetaldehyde measurements obtained with HPLC indicated traces of acetaldehyde in muscles of control animals, and *3*) enzyme-linked immunosorbent assays of homogenates from glucose-fed rats also showed some reactions above baseline (S Worrall, TJ Peters, and VR Preedy, unpublished observations, 2002).

The origins of this endogenous acetaldehyde remain unclear, but similar phenomena in the liver were also reported (27). It is likely that endogenous acetaldehyde production by intestinal bacteria may play a role (28–30). Acetaldehyde production during biochemical pathways could also contribute to adduct formation in tissues, including the cleavage of threonine to acetaldehyde and glycine (27, 31). Therefore, it would be reasonable to assume that there is a mechanism within the muscle cell for removing acetaldehyde. Indeed, studies showed that rat skeletal muscle contains cytochrome P450 (32), alcohol, and acetaldehyde dehydrogenase (33) and that human skeletal muscle contains acetaldehyde dehydrogenase isoforms 1, 2, and 5 (34). The presence of malondialdehyde-protein adducts in tissues of control animals may also reflect endogenous production and normal peroxidative activity within lipid domains (Figure 1).

To our knowledge, this is the first report on the generation of muscle malondialdehyde-protein adducts as a result of acute ethanol toxicity. However, previous research showed that malondialdehyde, measured as the molecular species per se, increased in muscle in several models of increased oxidative stress, including a combination of pyridostigmine and exercise (35, 36), ischemia-reperfusion injury (37, 38), aging (39), starvation (40), and endocrine abnormalities such as hyperthyroidism (41). In contrast, transgenic mice expressing human manganese superoxide dismutase showed reduced muscle malondialdehyde (42). Antioxidants, such as the alkaloid anisodamine and vitamin E, reduce malondialdehyde concentrations in muscle in ischemia-reperfusion injury (43, 44). It was suggested that measurements of malondialdehyde production might be useful in testing the efficiency of antioxidant treatment (45, 46). Also, changes in malondialdehyde concentrations were localized to specific subcellular organelles during oxidative stress, such as microsomes after exercise (47) and mitochondria during selenium deficiency (48). It was also suggested that malondialdehyde occurs in excessive amounts in liver disease caused by alcohol consumption (18, 19, 49).

In the present study, we chose to examine malondialdehyde-protein adducts with immunohistochemistry because direct assays for malondialdehyde in muscle may reflect non-hepatic or hepatic-derived plasma sources, for example as seen in muscle ischemia–reperfusion injury (50, 51). This approach also allowed us to study the distribution of the aldehyde-derived modifications. It appears that both subsarcoplasmic and intracellular malondialdehyde-protein adducts are formed after challenge with ethanol and acetaldehyde.

We also addressed the question of whether adduct formation is enhanced by pretreatment with cyanamide. Cyanamide is a potent inhibitor of aldehyde dehydrogenase. Previous studies reported suppression of low- and high-K_m (Michaelis constant) enzyme activities by 83% and 70%, respectively (52) and even complete inhibition of the low-K_m hepatic aldehyde dehydrogenase (53). The preferential adduct formation in plantaris muscle after acetaldehyde challenge may reflect, or may be responsible for, the enhanced susceptibility of this muscle to ethanol-induced damage. The increased amounts of malondialdehyde-protein adducts in plantaris muscle after cyanamide pretreatment, compared with ethanol exposure alone, suggests an increased reactive-oxygen-species load and enhanced oxidative stress at this site. Previously, decreased generation of reactive oxygen species in cyanamide + ethanol loading was found in isolated rat hepatocytes (54). This is at variance with our current findings in plantaris muscle, although in liver tissue we also observed no intensification of malondialdehyde-protein

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adducts in rats given cyanamide + ethanol compared with rats given ethanol alone.

Studies in the pancreas showed that cyanamide increases malondialdehyde production, compared with acute ethanol exposure alone (55). Apparently, different experimental conditions may produce different degrees of tissue-specific oxidative burden. A distinction should also be made between in vivo and in vitro studies. Previously, we showed a 25% decrease in muscle protein synthesis in vivo when ethanol was combined with cyanamide (56). Pretreatment with cyanamide exacerbated such effects, reducing the fractional rate of protein synthesis by 65% (56). Such phenomena could be associated with enhanced aldehyde-protein adduct formation. However, soleus muscle showed no potentiation of malondialdehyde-protein adducts (detectable with immunohistochemical methods) in rats treated with cyanamide + ethanol compared with ethanol-treated rats, but cyanamide + ethanol treatment suppressed protein synthesis in this muscle type, compared with ethanol alone (56).

Although acetaldehyde-protein adduct formation did not increase in soleus muscle as a consequence of either ethanol or cyanamide + ethanol treatments, there was a significant increase in plantaris muscle in the cyanamide + ethanol group. The differences in the responses of these 2 muscle types may reflect the general insensitivity of type I muscle to alcohol and the greater susceptibility of the plantaris muscle to ethanol toxicity (4, 5). Interestingly, the increase in acetaldehyde-protein adducts in the liver after cyanamide pretreatment was also of rather small magnitude. Although immunohistochemistry is not quantitative by nature, note that previous studies on cultured hepatocytes showed a doubling of a 37-kDa acetaldehyde-protein adduct with cyanamide and ethanol, compared with ethanol alone (57).

The pathogenic significance of adduct generation in muscle remains unknown, because so little is known about alcoholic myopathy, in contrast to alcoholic liver disease. However, if we use liver tissue as an example, certain functional consequences may be proposed. Both acetaldehyde- and malondialdehyde-protein adducts can induce immunologic reactions (22, 23). Human alcoholic patients with liver disease have elevated serum antibody titers against protein adducts, including hybrid adducts of malondialdehyde and acetaldehyde (58). Increased adduct formation may either contribute to or reflect biochemical and functional lesions in ethanol-exposed muscle (4, 5). Muscle membrane receptors could also be targeted by adduct formation. We recently showed reduced sarcolemmal dystrophin in ethanol-exposed muscle (R Rajendram, J Codd, J Salisbury, and VR Preedy, unpublished observations, 2002). Interference with the contractile apparatus could induce conformational and biophysical changes with concomitant impairment in force-generation and muscle strength, as observed previously in both experimental animals and humans (4, 5).

The clinical relevance of muscle adduct formation as a result of ethanol and acetaldehyde exposure also remains unclear at this time. Clinical studies showed that myopathy may occur in >50% of chronic alcoholics. Although acute myopathy is relatively rare, several lines of evidence have emphasized the adverse effects of periodic bouts of heavy drinking (1, 32, 59). Alcohol was also shown to be toxic to muscle in a dose-dependent manner, which supports a direct role for ethanol and its metabolites in the pathogenesis of muscle injury (60).

Taken together, the present findings indicate that ethanol administration results in the generation of aldehyde-derived protein adducts, which occur preferentially in type II muscle. These observations may reflect the increased susceptibility of anaerobic muscle to the toxic effects of alcohol. Cyanamide appears to exacerbate adduct formation, especially in this muscle type. Baseline adduct scores in control rats also appear to be lower in anaerobic muscle than in aerobic muscle. The present findings are relevant to studies on the pathogenesis of alcohol-induced myopathy.

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